Rapid Differentiation of Experimental Populations of Wheat for Heading Time in Response to Local Climatic Conditions

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● *Background and Aims* Dynamic management (DM) of genetic resources aims at maintaining genetic variability between different populations evolving under natural selection in contrasting environments. In 1984, this strategy was applied in a pilot experiment on wheat (*Triticum aestivum*). Spatio-temporal evolution of earliness and its components (partial vernalization sensitivity, daylength sensitivity and earliness *per se* that determines flowering time independently of environmental stimuli) was investigated in this multisite and long-term experiment.
● *Methods* Heading time of six populations from the tenth generation was evaluated under different vernalization and photoperiodic conditions.
● *Key Results* Although temporal evolution during ten generations was not significant, populations of generation 10 were genetically differentiated according to a north–south latitudinal trend for two components out of three: partial vernalization sensitivity and narrow-sense earliness.
● *Conclusions* It is concluded that local climatic conditions greatly influenced the evolution of population earliness, thus being a major factor of differentiation in the DM system. Accordingly, a substantial proportion (∼25 %) of genetic variance was distributed among populations, suggesting that diversity was on average conserved during evolution but was differently distributed by natural selection (and possibly drift). Earliness is a complex trait and each genetic factor is controlled by multiple homeoalleles; the next step will be to look for spatial divergence in allele frequencies.

Key words: Experimental populations, adaptation, differentiation, heading time, *Triticum aestivum*.

INTRODUCTION

The conservation of genetic diversity for crop species is classically carried out by storing seeds (or others propagules) of several thousand accessions per species in gene banks. Although such 'static' conservation allows preservation of a 'snapshot' of the genetic diversity present at the time of the collection (Frankel *et al.*, 1995), the preservation of individuals or populations *per se* may be of limited utility if continuous adaptation is required for growth under a changing environment such as pathogen, climatic or anthropic pressures (Simmonds, 1962; Henry *et al.*, 1991).

Trying to mimic natural population processes, dynamic management (DM) of populations has been proposed to ensure crop genetic diversity to fit the future needs of plant breeding (Henry *et al.*, 1991). This strategy for conservation of genetic resources aims at maintaining the evolutionary processes among a set of genetically diverse populations grown across generations in a network of different sites differing in pedo-climatic and biotic conditions. DM is expected to allow for adaptive changes within populations in response to local selection pressures, while simultaneously maintaining genetic variation on the whole.

The major role of natural selection in shaping the genetic differentiation between populations has been widely demonstrated using different approaches such as the quantitative trait locus (QTL) sign test (Rieseberg *et al.*, 2002) or the comparison of population differentiation

obtained using molecular markers and quantitative traits (Merilä and Crnokrak, 2001; McKay and Latta, 2002). At the species level, local adaptation arising from spatial heterogeneity of selection and low migration rates is a central mechanism for the maintenance of genetic variation for adaptive traits. At the population level, genotypic diversity can be lost by drift and selection, preventing future adaptation to new environmental conditions. Thus, characterizing the amount of migration and natural selection necessary to allow local adaptation but to prevent too much loss of diversity is a major issue in most conservation programmes of endangered species (Lythgoe, 1997; Frankham, 1999; Stockwell *et al.*, 2003). In DM programmes, it is necessary to characterize between-population differentiation for adaptive traits and identify the selection pressures involved in order to determine how much migration should be enforced between subpopulations.

Long-term experiments of natural selection on barley populations have been conducted in California since 1928 (Allard, 1988). The basic idea was that subsequent generations of cropping in bulk under natural selection of a hybrid population would lead to an improvement in agricultural fitness (Suneson, 1956). However, initial populations were not derived from the same genetic pool, preventing the study of spatial differentiation by genetic drift and selection, and the maintenance of genetic diversity on the whole. Such an experiment with different local populations set up from a single genetic pool started in 1984 on bread wheat (*Triticum aestivum*) (Henry *et al.*,

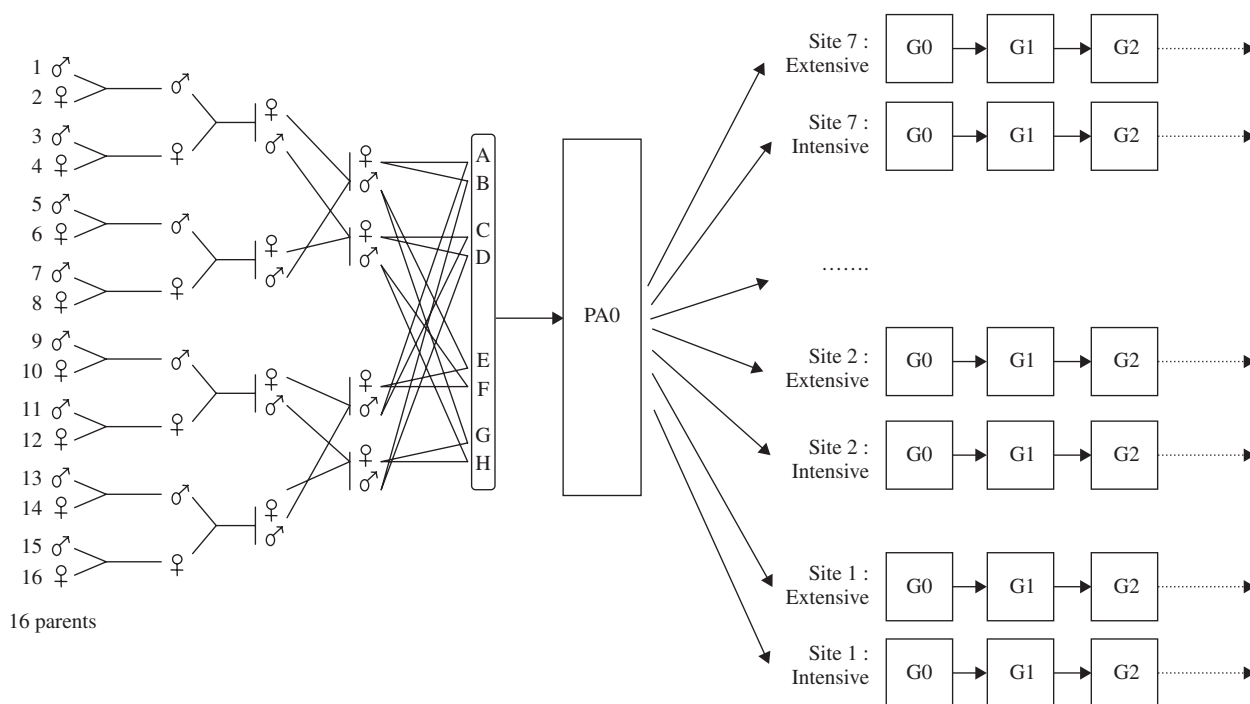* For correspondence. E-mail goldringer@moulon.inra.fr

FIG. 1. Formation of DM populations. First, the 16 parental lines were crossed with each other in a pyramidal way; then, the multiple hybrid was cultivated during three generations to increase seed number providing the PA0 starting population; finally, samples of PA0 seeds were distributed into seven sites of the French network to be cultivated under two growing conditions: extensive and intensive.

1991). After 6–10 years of cultivation, differentiation among populations was detected for many agro-morphological traits (David, 1992; Leboulc'h *et al.*, 1994; Goldringer *et al.*, 2001*a*), suggesting that local adaptation was in progress.

Heading date was one of these characters. The timing of first reproduction in plants has been shown to be controlled by the interaction of environmental and genetic factors (Bernier, 1992; Simpson *et al.*, 1999; Mouradov *et al.*, 2002). Temperature, through vernalization, and daylength are the main environmental conditions that synchronize flowering with the seasons, ensuring that the reproductive phase occurs during favourable conditions (Reeves and Coupland, 2000; Putterill, 2001; Henderson *et al.*, 2003). Heading date can be considered as an important adaptive trait. Indeed, initiating reproduction too early or too late in the life of an individual could have negative effects on its ability to produce progeny, on the survival of these progeny or on its subsequent reproductive performance for perennials. For example, global change, through the increase of summer temperatures in Europe, could select earlier flowering for some plant species, leading to populations with genotypes requiring less vernalization than previously (Etterson and Shaw, 2001). Heading time in many plant species from temperate climates is a complex character controlled by three major factors: (*a*) vernalization requirement; (*b*) photoperiod sensitivity; and (*c*) earliness *per se* which is defined as earliness in fully vernalized plants under long-day conditions (Kato and Yamagata, 1988).

Here spatio-temporal differentiation for earliness and its components is studied among a set of experimental wheat populations maintained under DM in a multisite network for ten generations. The objective of the present study was to test the hypothesis that genetic differentiation for earliness was due to adaptation to local climatic conditions, hence providing arguments that diversifying selection was a major contributor to the maintenance of genetic variation under dynamic management. For this, heading date was studied in terms of its components to identify environmental factors possibly responsible for the differentiation. This is also the first step for further studies on the genetic basis involved in this differentiation.

MATERIALS AND METHODS

Development of the DM populations

Sixteen parental lines of *T. aestivum* L. representing a wide genetic basis were initially crossed using a pyramidal design (Thomas *et al.*, 1991). The hybrid seeds obtained were then sown and bulk harvested for three generations to increase seed amount. This composite cross population (called PA0) is the initial population of the DM programme. In 1984, seed samples of PA0 were distributed to seven sites of a French multilocation experimental network (Figs 1 and 2; for more details, see Henry *et al.*, 1991). Two methods of cultivation were used in each site: an intensive farming method, where wheat was grown under the conventional method for the area, and an extensive farming method, with one-third of the nitrogen fertilizer used in the intensive method and no fungicide treatments. Hence in the DM system, a local population is

F I G . 2. Network for the dynamic management of the wheat genetic resources programme (DGER of the French Agriculture Ministry, INRA, INA-PG and BRG). The six populations studied for their heading time are indicated in bold; E and I stand for extensive and intensive cropping system, respectively. DGER, Direction Générale de l'Enseignement et de la Recherche; INRA, Institut National de la Recherche Agronomique; INA-PG, Institut National Agronomique Paris-Grignon; BRG, Bureau des Ressources Génétiques.

fully described by its site of cultivation and its farming method. Since 1984, each population has been cultivated every year, without any conscious human selection. A new generation was constituted by plants grown at the same location under the same conditions and stemmed from a set of seeds sampled within the harvest of the preceding generation. A $100 \, \text{m}^2$ cultivated area was generally used to obtain approx. 10 000–15 000 plants per population. All local populations were isolated from each other and from neighbouring crops to avoid unwanted cross-pollination.

Plant material used to study the developmental parameters

The parents and the initial population were studied together with six DM populations after ten generations of dynamic management and originating from four locations of the network: Toulouse (TO; 43·4°N, 1·3°E), Rennes (LR; 48·1°N, 1·4°W), Le Moulon (LM; 48·4°N, 2·1°E) and Vervins (VV; 49·5°N, 3·5°E) (Fig. 2). These sites show contrasting climatic conditions in terms of temperature

and/or daylength (Fig. 3). Given that at each location, both populations [cultivated under an intensive (I) and extensive (E) farming method] were not always available, both the populations of Toulouse (TOI and TOE) and Rennes (LRI and LRE), but only one population for Le Moulon and Vervins (LME and VVI, respectively) were studied.

Sensitivity to partial vernalization, narrow-sense earliness and daylength sensitivity were estimated for the 16 parental lines, 16 single-seed descent lines derived from the initial population (PA0) and 84 inbred families from the six DM populations: 16, 11, 15, 12, 16 and 14 families from VVI, LME, LRE, LRI, TOE and TOI, respectively (Fig. 1). Inbred families were derived by two selfing generations from seeds sampled in the stock of the corresponding populations. It was assumed that due to the mainly selfing mating system of these populations (outcrossing rate = 2–9 %; Enjalbert and David, 2000), the families were close to fixation. Each genotype (parental line, PA0 SSD line and inbred family for the DM populations) was represented by approx. 100 genetically homogenous selfed seeds.
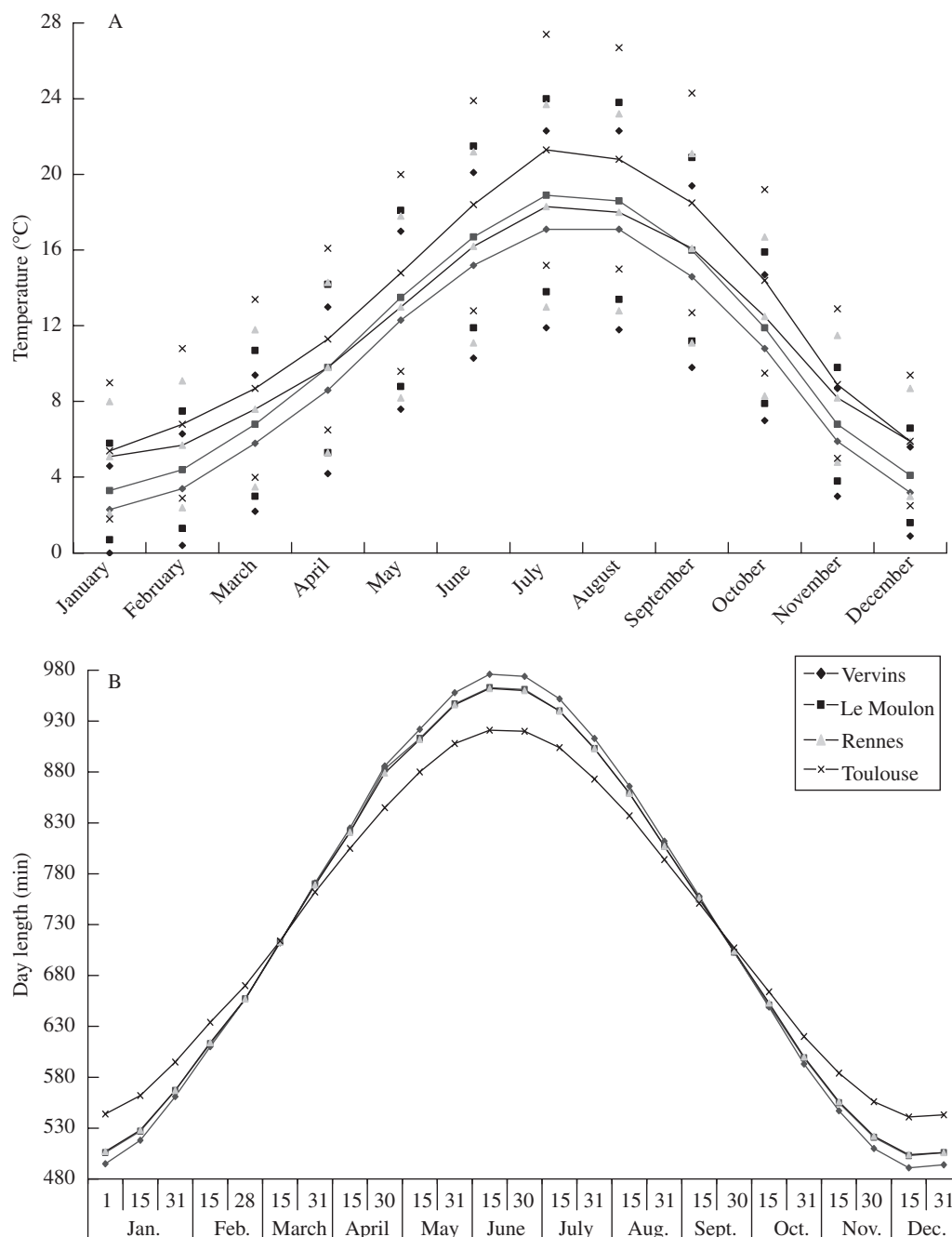
FIG. 3. Climate diagrams (A, temperature; B, daylength) for the four studied sites. The temperature diagram was obtained from daily temperatures averaged over 30 years (continuous lines) by the national meteorological office. Maximum and minimum temperatures per month are also indicated for each site.

Experimental design

All genotypes were grown and evaluated together in two experiments replicated in two years (2002, 2003) at Le Moulon: a field trial and a garden nursery. In the field trial, all the genotypes were sown on 25–26 October in 2001 and 30 October in 2002, in a two-replicate complete block design with 1·20 m length single-row plots using 20 seeds per plot and per genotype. Since the parents were not evaluated in 2002, only 100 genotypes were evaluated (16 PA0 + 84 DM, i.e. 200 plots) while all the

116 genotypes were evaluated in 2003 (232 plots). For each genotype, the date of heading (denoted HdField) was recorded when half of the ears on the plot had emerged from the flag leaf. Given that plants were subject to cold winter temperatures, it was assumed that the vernalization requirement was satisfied in this experiment, and that different flowering dates did not reflect different cold requirements. Conversely, as daylength was increasing after the vernalization period, it was assumed that heading date for an autumnal sowing in Northern France reflects

photoperiod sensitivity (Masle *et al.*, 1989) and earliness *per se*.

In the nursery experiment, all the genotypes were grown under three cold treatments: no vernalization, partial vernalization (4 weeks of vernalization) and complete vernalization (6 weeks in 2002 / 8 weeks in 2003). For 'vernalization' treatments, seeds (seven per genotype per treatment, i.e. $116 \times 7 = 812$ seeds per treatment) were germinated in pots at room temperature. Then, at one-leaf stage, seedlings were grown for either 4 weeks, or for 6 or 8 weeks with constant temperature ($4\,°C$) in a cold growth chamber with a 12 h daylength regime. Vernalized seedlings were transplanted in the garden nursery. For the 'no vernalization' treatment, five seeds per genotype (i.e. $116 \times 5 = 580$ seeds in total) were sown directly in the nursery. Sowing time was adjusted to the treatment duration so that the three treatments were completed at the same time (mid-April). For each plant and treatment, ear emergence day of the main tiller was recorded as the day of heading and denoted HdNV, HdV4, and HdV6 or HdV8 for no vernalization, partial and complete vernalization treatment, respectively. Given that all seedlings were planted out under long-day conditions for the nursery experiment, it was assumed that the daylength requirement was satisfied in this experiment, and that different flowering dates did not reflect different sensitivities. Conversely, the comparison of heading date between treatments will provide information about vernalization requirement satisfaction.

Trait measurement and transformation into developmental variables

First, each heading date was transformed into the sum of degree-days ($°Cd$; thermal time with $0\,°C$ basis) from germination to heading. To limit within-genotype environmental variation for earliness, means per genotype were calculated for each treatment and each experiment.

A qualitative variable based on the behaviour of the genotypes in the 'no vernalization' treatment described the growth habit: following Worland (1996), a genotype was defined as a 'spring wheat' if it headed without vernalization before the end of the experiment (4 months); otherwise it was defined as a 'winter wheat'. Average heading time was split up into three components per genotype: sensitivity to partial vernalization, narrow-sense earliness and daylength sensitivity. Partial vernalization sensitivity (PVS) was determined as the quantitative response to cold temperature exposure. It was calculated as the difference between the mean heading time in the partially vernalized treatment and the mean heading time in the fully vernalized treatment: PVS = HdV4 − HdV6 or PVS = HdV4 − HdV8. Narrow-sense earliness was assessed through the mean heading time measured in the complete vernalization treatment (HdV6 or HdV8), since vernalization requirements were satisfied and long-day conditions were met at planting time. The third parameter, daylength sensitivity (DLS), was estimated as the difference between heading time in the field

experiment (reflecting both daylength sensitivity and narrow-sense earliness) and heading time in the complete vernalization treatment and long-day conditions HdV6 or HdV8 (reflecting narrow-sense earliness alone): DLS = HdField − HdV6 or DLS = HdField − HdV8.

Data analysis

Analyses of variance were performed either on the whole data set (years 2002 and 2003) or on sub-samples, for HdField, and the three component variables PVS, HdV6/HdV8 and DLS using the GLM procedure of the SAS Software package (SAS Institute 2000).

Preliminary analysis. First, it was determined whether the mean heading time after 6 weeks vernalization in 2002 (HdV6) and after 8 weeks in 2003 (HdV8) could be considered as a single variable (HdV6/V8) representing the response after fully vernalized treatment. For that, in the same column the data of the two years (HdV6 with HdV8 for the response after fully vernalized treatment) were pooled and the following model was applied:

$$Y_{ijk} = \mu + yr_i + pop_j + (yr \times pop)_{ij} + geno(pop)_{jk} + R_{ijk} \qquad (1)$$

with *yr* the fixed year effect, *pop* the random population effect, *geno* the random genotype effect and *R* the random residual. The *yr*, *pop* and *yr* × *pop* effects effects were tested on the residual, while the *pop* effect was tested on the *geno*(*pop*) mean square. The year effect was highly significant ($P < 0.001$) for HdField, PVS and DLS, and significant with $P = 0.03$ for HdV6/V8, whereas the interaction *yr* × *pop* was never significant. Hence, the behaviour of HdV6/V8, though influenced by the year of experimentation (genotypes headed $12.5\,°Cd$, i.e. about half a day, earlier in V8 than in V6), was rather similar to that of any other variable. Thus HdV6/V8 was considered instead of both HdV6 and HdV8, increasing the power of the statistical analyses. For simplification in the following, the interaction *yr* × *pop* was removed from model (1) analysis of variance (ANOVA) since it was never significant; the corresponding model will be called (1′).

In order to choose an initial sample for studying temporal evolution, parents and PA0 were compared using model (1′) (with *pop* = 'par' or *pop* = 'PA0'). This effect was never significant at the 5 % level. The same result was observed for growth habit type composition. This led to PA0 being chosen as the initial state since it is the population from which the DM experiment started (Fig. 1).

Analysis of temporal and spatial differentiation. Temporal differentiation was studied by comparing the six DM populations (10th generation) with PA0. Population means of the six DM populations adjusted for the year effect using ANOVA model (1′) were compared with the corresponding adjusted PA0 mean with a Dunnett test (procedure for comparing several treatments with a control;

Dunnett, 1955) using the *geno*(*pop*) mean square as the residual for the test. This is a multiple comparison procedure which holds the maximum experimentwise error rate under α (here α = 5 %). Spatial differentiation was studied based on the six DM populations only. To study the influence of local selection due to eco-geographical conditions or farming method in the cultivation sites of the DM populations, two different ANOVA models derived from model (1′) had to be run. In model (2a), which was only applied on the Toulouse and Rennes extensive and intensive populations, a farming method effect (*farm*) could be tested for properly; but the local eco-geographical conditions effect (*site*) was not considered informative since the analysis was restricted to only two of the four sites.

$$Y_{ijkl} = \mu + yr_i + farm_j + site_k$$
$$+ geno(site \times farm)_{jkl} + R_{ijkl} \qquad (2a)$$

In model (2b), a site effect conditional to each farming method was tested for using the six populations; farming method was not assessed properly here since different sites were taken into account for each of the two farming methods.

$$Y_{ijkl} = \mu + yr_i + farm_j + site(farm)_{jk}$$
$$+ geno(site \times farm)_{jkl} + R_{ijkl} \qquad (2b)$$

Note that the random genotype effects from models 2a and 2b [*geno*(*site × farm*)] and from models 1 and 1′ [*geno*(*pop*)] are equivalent. Farming method and site effects were tested using the *geno*(*site × farm*) mean square as a denominator.

For the site effect in model (2b), when the Fisher test was significant at the 5 % level, least square means pairs of populations within farming method were compared. To take into account the multiple level of comparison, the Bonferroni correction was used and the level of significance for each comparison (comparisonwise error rate) was set to a value of α/*c*, with *c* the number of comparisons (here *c* = 3). It ensures the maximum experimentwise error rate to be kept under α. Habit type frequencies were compared between all DM populations using Fisher's exact test with significance at the 5 % level.

Between- and within-population genetic variances were estimated from model (1′) applied to the six DM populations of the 10th generation using the VARCOMP procedure of SAS software (REML method, SAS Institute 2000).

RESULTS

Phenotypic variability and relationships between traits

Genotype means estimated from ANOVA model (1′) on the whole data set were used to study relationships between traits. HdField was highly correlated with HdV6/V8 (*r* = 0·560) (Fig. 4A) and with DLS (*r* = 0·54), and to a lesser extent with HdV4 (*r* = 0·28), whereas it was not correlated with PVS (Table 1). The two first correlations did not vary much among populations (Table 1). The correlation between HdField and HdV6/V8 was always greater than 0·4 except in Toulouse intensive, although it was significant only in five cases due to the small sample sizes. Similarly, the correlation between HdField and DLS was always larger than 0·45 except for Vervins intensive. Conversely, correlations between HdField and HdV4, or HdField and PVS were not consistent among populations and they were seldom significant. This indicated that as expected in the conditions of the field experiment, the time to heading of the genotypes was determined by both their narrow-sense earliness and their photoperiod sensitivity. The relationship between earliness under partial vernalization (HdV4) and under complete vernalization (HdV6/V8) illustrates the threshold effect of vernalization (Fig. 4B). Though HdV4 was highly correlated to HdV6/V8 at the global level (*r* = 0·83, *P* < 0·0001) as well as in all populations (Table 1), genotypes could be divided into two groups: those having about the same values for HdV4 and for HdV6/V8 (close to the *y* = *x* line), and those clearly above (HdV4 >1250 °Cd). This suggested that genotypes of the first category had their vernalization requirements satisfied with only 4 weeks at low temperatures (low values of PVS). Genotypes of the second category headed later with partial vernalization than with a complete vernalization treatment, suggesting that their vernalization requirements were not satisfied with only 4 weeks of cold treatment (high values of PVS). While some of these genotypes stemmed from the parents, PA0, LME, LRI or TOI, most of them belonged to the Vervins population, the most cold-adapted population. A significant negative correlation (*r* = −0·48, *P* < 0·0001) was reported between PVS and DLS overall (Fig. 4C), but also within populations, except within Rennes extensive, Vervins intensive and the parents, where it was negative but not significant (Table 1), indicating that the global negative relationship was not due to differences between populations. Rather, the correlation was not consistently found when considering separately the two winter and spring genotypes, no correlation being found within the later category (Table 1). The correlations between PVS and other traits assessed in the group of spring genotypes were always close to zero probably due to the lack of variation for PVS within spring wheats (Table 1). Indeed, the group of genotypes with high vernalization requirements (high PVS) had rather low DLS, while genotypes with very low PVS (statisfied with 4 weeks of cold treatment) were the most sensitive to daylength (Fig. 4C).

Temporal differentiation for DM populations

For the four traits studied (HdField, HdV6/V8, PVS and DLS), the comparison of the mean value between each of the six DM populations and PA0 yielded no significant effect. Populations Vervins intensive and Toulouse extensive were often the most different from PA0, and the PA0 mean appeared intermediate between the DM population means for all traits (Fig. 5), revealing no
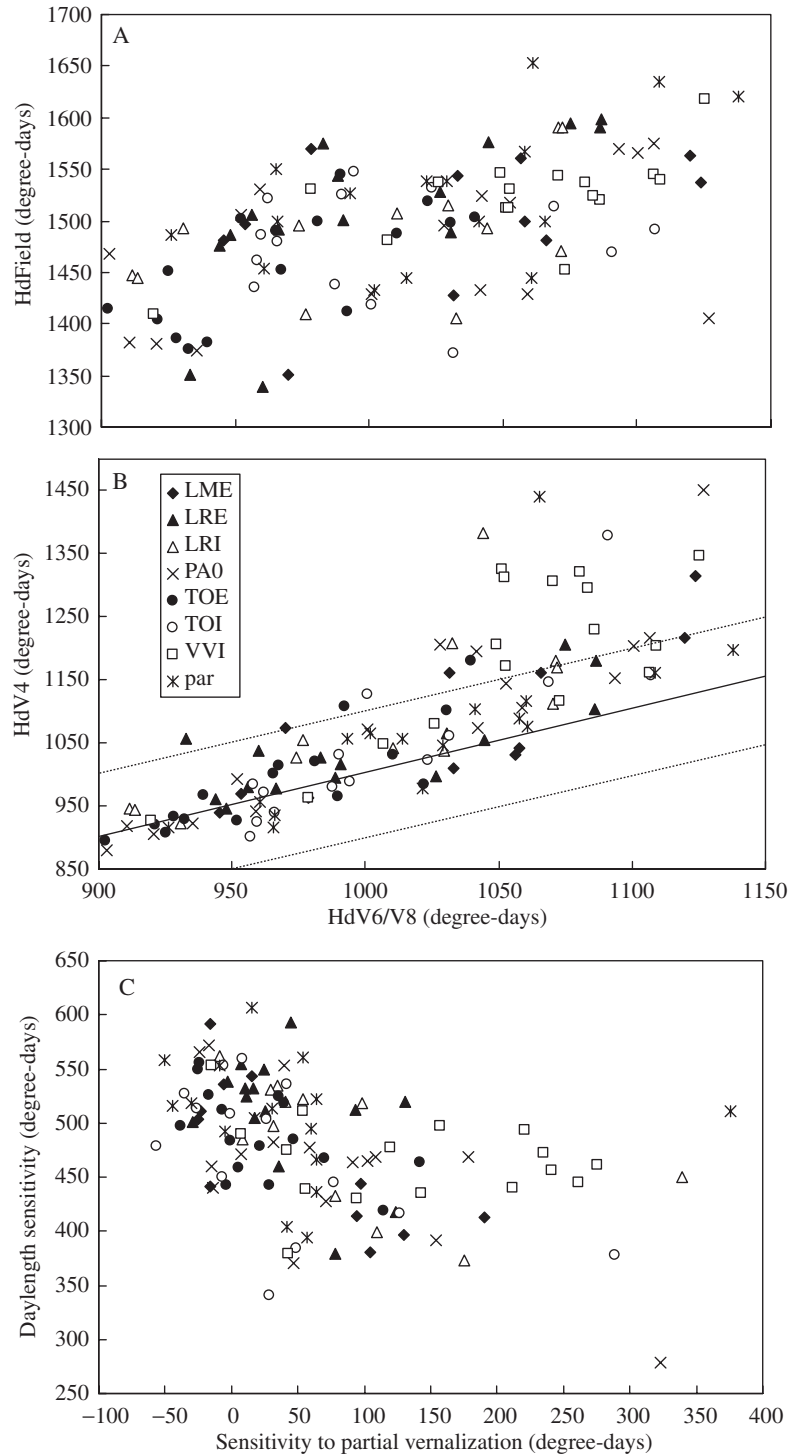
F I G . 4. Relationship between genotype means adjusted to year effect in ANOVA model (1′) for different traits: (A) time to heading in field conditions (HdField) and time to heading with 6/8 weeks of low temperatures (HdV6/V8, 'complete vernalization'); (B) time to heading with 4 weeks of vernalization (HdV4, 'partial vernalization') and HdV6/V8; (C) daylength sensitivity (DLS) and sentivity to partial vernalization (PVS). Each point represents one genotype. TOE, Toulouse extensive; TOI, Toulouse intensive. LME, Le Moulon extensive; LRE, Rennes extensive; LRI, Rennes intensive; VVI, Vervins intensive. The $y = x$ line and lines delimiting the range of variation of $\pm 100\,°$Cd around the $y = x$ line were drawn on (B).

significant temporal differentiation. Similarly, for the frequencies within populations of the two growth habit types ('winter' and 'spring'), the comparison between each of the six DM populations and PA0 using Fisher's exact test was never significant. Neither the increase in spring type frequency in Rennes extensive and Toulouse intensive nor the increase in winter type frequency in Vervins intensive (Fig. 6) remained significant.

TABLE 1. *Correlations of genotypic means adjusted to year effect [estimated from ANOVA model (1′)] between traits: time to heading in field conditions (HdField), time to heading with 6/8 weeks of low temperatures (HdV6/V8, 'complete vernalization'), time to heading with 4 weeks of vernalization (HdV4, 'partial vernalization'), sentivity to partial vernalization (PVS) and daylength sensitivity (DLS)*

Level	n	r(HdField − HdV6/V8)	r(HdField − HdV4)	r(HdField − PVS)	r(HdField − DLS)	r(HdV4−HdV6/V8)	r(DLS − PVS)
Whole set	116	0·560 (<0·0001)	0·282 (0·002)	0·031 (0·744)	0·545 (<0·0001)	0·828 (<0·0001)	−0·479 (<0·0001)
Winter type	72	0·597 (<0·0001)	0·218 (0·066)	−0·067 (0·578)	0·673 (<0·0001)	0·694 (<0·0001)	−0·325 (0·005)
Spring type	44	0·704 (<0·0001)	0·581 (<0·0001)	0·048 (0·757)	0·764 (<0·0001)	0·865 (<0·0001)	−0·044 (0·777)
Parents	16	0·569 (0·021)	0·174 (0·518)	−0·097 (0·719)	0·617 (0·011)	0·732 (0·001)	−0·496 (0·051)
PA0	16	0·496 (0·050)	0·266 (0·320)	0·040 (0·883)	0·450 (0·080)	0·915 (<0·0001)	−0·709 (0·002)
VVI	16	0·683 (0·004)	0·597 (0·015)	0·443 (0·086)	0·251 (0·348)	0·776 (0·0004)	−0·172 (0·523)
LME	11	0·416 (0·203)	−0·008 (0·982)	−0·355 (0·283)	0·579 (0·062)	0·829 (0·002)	−0·747 (0·006)
LRI	12	0·516 (0·086)	0·137 (0·671)	−0·138 (0·670)	0·479 (0·115)	0·766 (0·004)	−0·585 (0·046)
LRE	15	0·740 (0·002)	0·397 (0·143)	−0·201 (0·472)	0·747 (0·001)	0·790 (0·003)	−0·433 (0·107)
TOI	14	0·030 (0·918)	−0·062 (0·833)	−0·108 (0·713)	0·687 (0·007)	0·853 (<0·0001)	−0·551 (0·041)
TOE	16	0·697 (0·003)	0·366 (0·163)	−0·001 (0·997)	0·636 (0·008)	0·845 (<0·0001)	−0·553 (0·026)

Correlations are given for the whole set of genotypes, within both groups of winter and spring types and within each population. *P*-values are given in parentheses.

Spatial differentiation between DM populations

Spatial differentiation was studied, based on the six DM populations at the 10th generation. In model (2a), the farming method was never significant (Table 2). While for PVS, the intensive populations seemed to have higher values (but not significant, *P* = 0·15), and for DLS, lower values (not significant, *P* = 0·17), the farming method did not have a constant effect in all sites and over all variables. Conversely, the 'site within farming method' effect in model (2b) was significant for HdField, HdV6/V8 and PVS (*P* = 0·036, *P* = 0·003 and *P* = 0·018, respectively), but not significant for DLS (*P* = 0·41) (Table 2).

Except for DLS, for which no significant differentiation was observed, southern populations (Toulouse intensive and Toulouse extensive) were always the earliest heading populations and needed less vernalization, whereas the Vervins northern population was the latest heading with highest vernalization requirements, Le Moulon and Rennes populations being intermediates (Fig. 5). Hence, population field heading date, narrow-sense earliness and sensitivity to partial vernalization were differentiated according to the latitude of their cultivation sites.

An analysis of wheat type frequencies led to the same conclusion (Fig. 6). Although each wheat type could be found in each population, growth habit type frequencies were significantly different between DM populations (*P* = 0·024 with global Fisher's exact test). The frequency of winter type was higher in the Vervins population (87·5 %), as compared with Toulouse intensive, Toulouse extensive and Rennes extensive populations where spring type was dominant or at least equivalent (64·3, 50·0 and 60·0 %, respectively) (probabilities with Fisher's exact test: Vervins intensive vs. Toulouse intensive. *P* = 0·004; Vervins intensive vs. Toulouse extensive, *P* = 0·008; Vervins intensive vs. Rennes extensive, *P* = 0·004).

Spatial differentiation between populations was also associated with the genetic variance for HdField, PVS, HdV6/V8 and DLS (Table 3). For PVS and HdV6/V8, the between-DM populations represented about a quarter of

the total genetic variation in DM populations at generation 10, for HdField it was 9 %, whereas it was close to zero for DLS, which is consistent with the results of spatial differentiation.

DISCUSSION

Few studies have split up earliness into its three components and shown genetic diversity and spatio-temporal evolution for these characters (Cooper, 1963; Loskutov, 2001; Boudry *et al.*, 2002). Assessing the components of earliness in addition to measuring heading time in the field provides a means of identifying the environmental factors that have influenced selection on earliness in different areas. While the time to heading measured in the field mainly depends on the environmental conditions of the location, particularly winter and spring temperature and photoperiod, this is not the case for developmental components. In this experiment, as shown on the temperature diagram (Fig. 3A), vernalizing temperatures occur from November to April at Le Moulon. As a consequence, time to heading measured at this site, instead of reflecting vernalization requirement, would account for both narrow-sense earliness and daylength sensitivity (Fig. 4A).

Here, it was shown that ten generations of cultivation in highly contrasted environments resulted in genetic differentiation of experimental populations of wheat for field heading time and for two out of three developmental parameters: narrow-sense earliness and sensitivity to partial vernalization or frequency of growth habit types ('winter' and 'spring' types). Since the sensitivity to partial vernalization and the growth habit type of a genotype both are linked to its vernalization requirements, and because both traits have similar responses in this study, they are referred to by the term 'vernalization requirements' in the following discussion. While spatial structure was marked, no significant temporal evolution between generation 0 and 10 was revealed for these components.
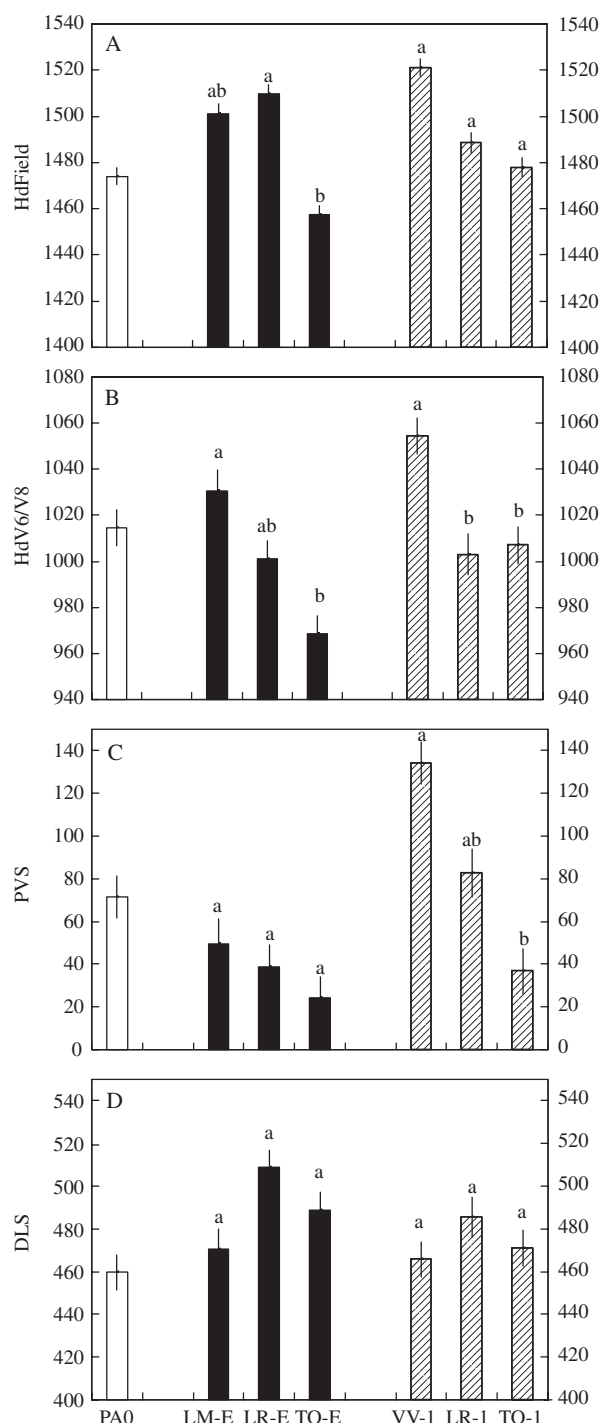
FIG. 5. Bar chart of population means adjusted to year effect (with s.e.) for (A) HdField, (B) HdV6/V8, (C) PVS and (D) DLS. Except for PA0, populations are grouped according to their farming method (E, extensive; I, intensive) and ordered by decreasing latitude. Letters correspond to multiple comparisons between populations within each farming method (see Materials and methods for more details). HdField, heading date in field experiment; HdV6/V8, heading date in 'complete vernalization' treatment; PVS, sensitivity to a 'partial vernalization' treatment; dates were transformed into sums of degree-days (thermal time with a 0 °C basis) from germination to heading (see Experimental design for more details). PA0, starting population; LME, Le Moulon extensive; LRE, Rennes extensive; TOE, Toulouse extensive; VVI, Vervins intensive; LRI, Rennes intensive; TOI, Toulouse intensive.
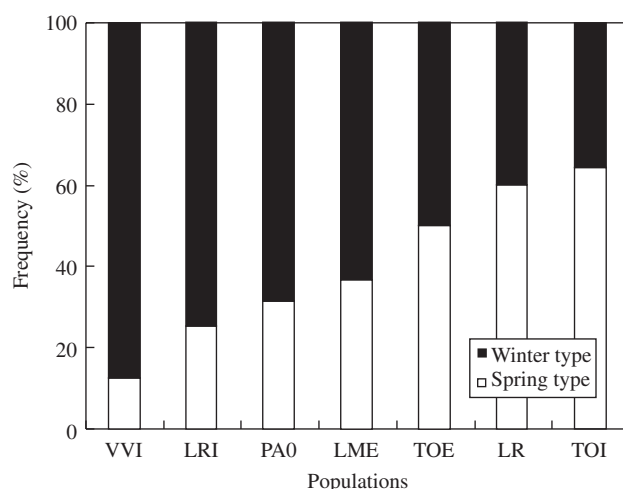

FIG. 6. Frequencies of spring and winter wheat types in the DM populations. TOE, Toulouse extensive; TOI, Toulouse intensive; LME, Le Moulon extensive; LRE, Rennes extensive; LRI, Rennes intensive; VVI, Vervins intensive.

Since divergent selection shifts the genetic mean of North and South populations to extreme values, genetic differentiation between DM populations is stronger than genetic differentiation between the starting population (intermediate phenotype) and each DM population. It should be noted that due to the sample sizes used, the diffentiation between populations might be underestimated. As a consequence of spatial differentiation, a variable (9, 23 and 27 %, respectively) proportion of the genetic variance at generation 10 was distributed among populations for field heading time, sensitivity to partial vernalization and narrow-sense earliness, respectively. Due to small sample sizes, estimates of genetic variance have to be considered with caution in this study. However, for field heading time, vernalization requirements and narrow-sense earliness, the spatial differentiation between populations and the intermediate position of the initial population (PA0) among the 10th generation DM populations suggested that the original genetic variability was conserved during evolution but differently distributed among populations due to drift and natural selection.

Similar evolution has been shown for the frequency of genes for specific resistance to powdery mildew *Erysiphe graminis* f. sp. *tritici* (Le Boulc'h *et al.*, 1994; Paillard *et al.*, 2000*a*, *b*) or to eyespot *Pseudocercosporella herpotrichoides* (Fron.) Deighton (Goldringer *et al.*, 2001*a*). As wheat is a self-pollinating species, a population size of 10 000 individuals corresponds to about 5000 individuals of a panmictic population. Such a demographic size should limit genetic drift. Yet, using molecular markers to assess temporal variations in allele frequencies, Goldringer *et al.* (2001*b*) found that effective population sizes in the DM system were lower than expected as a result of drift alone in populations of such size. Heritable variation in reproductive success, i.e. natural selection, was suspected to reinforce the local reduction of population diversity by genetic drift. The results confirm the extent of heterogeneous selection between populations. The

TABLE 2. *ANOVA tables with models (2a) (on four populations at generation 10) and (2b) (on six populations at generation 10) testing for spatial differentiation for HdField (heading time measured in the field), HdV6/V8 (narrow-sense earliness), PVS (sensitivity to partial vernalization) and DLS (daylength sensitivity)*

Trait	Source of variation	d.f.	Sum of squares	Mean square	F-value	P-value	Source of variation	d.f.	Sum of squares	Mean square	F-value	P-value
			Model (2a)						Model (2b)			
HdField	Year	1	227 353	227 353	604·26	<0·0001	Year	1	319 117	319 117	716·56	<0·0001
	Farm	1	12	12	0·00	0·969	Farm	1	1799	1799	0·25	0·618
	Site	1	31 227	31 227	3·99	0·051	Site(farm)	4	77 880	19 470	2·71	0·036
	Geno(site × farm)	54	422 110	7817	20·78	<0·0001	Geno(site × farm)	78	560 170	7182	16·13	<0·0001
	Error	56	21 070	376			Error	83	36 964	445		
HdV6/V8	Year	1	480	480	0·31	0·577	Year	1	3060	3060	1·62	0·206
	Farm	1	12 574	12 574	2·33	0·132	Farm	1	19 486	19 486	3·44	0·067
	Site	1	7247	7247	1·34	0·251	Site(farm)	4	99 241	24 810	4·38	0·003
	Geno(site × farm)	54	291 014	5389	3·52	<0·0001	Geno(site × farm)	78	441 460	5660	3·00	<0·0001
	Error	56	85 657	1530			Error	83	156 359	1884		
PVS	Year	1	16 011	16 011	5·72	0·020	Year	1	40 053	40 053	13·06	0·0005
	Farm	1	20 294	20 294	2·10	0·153	Farm	1	91 803	91 803	7·72	0·007
	Site	1	23 551	23 551	2·37	0·130	Site(farm)	4	150 199	37 550	3·16	0·018
	Geno(site × farm)	54	537 376	9951	3·55	<0·0001	Geno(site × farm)	78	927 928	11 897	3·88	<0·0001
	Error	56	156 868	2801			Error	83	254 468	3066		
DLS	Year	1	207 019	207 019	111·18	<0·0001	Year	1	387 360	387 360	186·35	<0·0001
	Farm	1	11 852	11 852	1·93	0·170	Farm	1	9871	9871	1·62	0·207
	Site	1	8481	8481	1·38	0·245	Site(farm)	4	24 318	6079	1·00	0·414
	Geno(site × farm)	54	331 541	6140	3·30	<0·0001	Geno(site × farm)	78	475 664	6098	2·93	<0·0001
	Error	56	104 271	1862			Error	83	172 533	2079		

TABLE 3. *Within- and between-DM populations genetic variances (V_G) and their standard deviations for HdField (heading time measured in the field), PVS (sensitivity to partial vernalization), DLS (daylength sensitivity) and HdV6/V8 (narrow-sense earliness)*

10th generation DM populations	HdField		PVS		HdV6/V8		DLS	
	V_G (% total)	s.d.	V_G (% total)	s.d.	V_G (% total)	s.d.	V_G (% total)	s.d.
Between-populations	323 (9%)	352	1329 (23%)	1086	683 (27%)	549	63 (3%)	(173)
Within-populations	3357 (91%)	572	4405 (77%)	979	1884 (73%)	475	2001 (97%)	(511)
Total	3680	658	5734	1444	2567	717	2064	(526)

comparison of population differentiation indices obtained from molecular markers (*Fst*) and from quantitative traits (*Qst*) [according to McKay and Latta (2002)] confirmed the role of natural selection in shaping genetic differentiation between the DM populations. Whatever the flowering components considered, *Qst* (0·09−0·27) was higher than *Fst* estimated on 30 restriction fragment length polymorphism (RFLP) loci among Le Moulon, Rennes and Toulouse at generation 10 (*Fst* = 0·04 in Enjalbert *et al.*, 1999). For field heading time (measured at Le Moulon 48·4°N 2·1°E), sensitivity to partial vernalization, population composition in growth habit types and narrow-sense earliness, spatial differentiation consisted of a North–South trend through the studied area (France). Similar trends for vernalization requirement have been shown for several other plant species (e.g. *Beta vulgaris* ssp. *maritima*, Boudry *et al.*, 2002; *Avena* spp., Loskutov, 2001; *Lolium* sp., Cooper, 1952). Independent of the farming method, early heading and low vernalization requirements were preferentially selected in southern populations (Toulouse), while late heading and strong vernalization requirements

were selected in northern population (Vervins). This result indicated that geographical location with its climatic and ecological/pedological characteristics was probably more important than the cropping system for selecting heading time components. While it is possible that farming method (conditions such as sowing density, nitrogen supply, disease level, etc.) might influence the evolution of earliness components in each site, the results failed to show differences among sites due to farming methods.

Variation in vernalization requirement, which is also usually related to cold resistance during winter, can be associated with climatic differences between growing sites. In the northern part of France, the winter is rather cold and long, with freezing conditions late in spring time (Fig. 3A). Winter genotypes, which require a long vernalization period and are more resistant to cold temperatures, are expected to exhibit a better adaptation to such conditions (Iwaki *et al.*, 2000, 2001). Even though the distribution of spring and winter types could be related to climatic conditions, as selection pressure would favour the establishment of spring types in southern populations of

France and winter types in northern populations, some 'winter' genotypes were found in one population in Toulouse and 'spring' genotypes were also encountered in the Vervins population. After ten generations, it is likely that populations did not reach their genetic equilibrium, and that all unadapted genotypes had not yet been eliminated by natural selection, explaining why they were still found at low frequency in DM populations. Whereas selfing allows efficient selection within a population, permitting the production of valuable individuals when there is no depression of inbreeding, outcrossing recreates genotypic variance within a population. It probably delayed the effect of natural selection on genetic differentiation, but also provided a better management of variability for further improvement of DM populations.

For narrow-sense earliness, genotypes of populations from southern areas were genetically earlier. This result can also be explained by adaptation to warm and dry summers. Early growth ensures completion of the life cycle before heat and water stresses appear. In these climatic conditions, seed filling of late maturing plants may actually be hindered, whereas in the North these stresses seldom occur and late-maturing plants can accumulate more dry matter (Le Boulc'h *et al*., 1994; Goldringer *et al*., 2001*a*). Furthermore, populations of Le Moulon and Rennes had an intermediate response for the two components of earliness. This could be interpreted as a consequence of climatic regimes in which seasons are less marked than in southern and northern areas (Fig. 3). For instance, the temperature during winter and early spring (until April) is higher in Rennes than in Le Moulon, with a lower number of frost days in Rennes (38 vs. 48, daily temperatures averaged over 30 years), whereas from April to August the temperature is higher in Le Moulon (Fig. 3A). These climatic factors in Rennes and Le Moulon may combine so as to lead to a similar response for earliness.

As recorded in many studies (Worland, 1996; Law, 2000; Shindo *et al*., 2003), wheat is a daylength-sensitive plant. Most varieties require a definite period of long days for heading. Thus, daylength sensitivity seems to be an important component of wheat earliness. In northern France, photoperiod-sensitive plants should be selected for their ability to avoid late frost days. Conversely, in southern areas, daylength insensitivity should be selected to avoid early water stress. For example, Olmsted (1945) has demonstrated ecotypic differentiation in *Bouteloua curtipendula*, with flowering depending on the daylength encountered in the original sites of the plants. However, no significant differences have been observed among DM populations of wheat (generation 10) in this study. Moreover, a global significant negative correlation was found between daylength sensitivity and sensitivity to partial vernalization, whereas a positive relationship would be expected under the hypotheses derived above. This suggested that plants develop either one or the other sensivity mechanism with priority given to vernalization requirements, which are stimulated earlier in the development of wheat. Similarly to the study of Karsai *et al*. (2001) on barley, the negative correlation disappeared when considering the spring genotypes separately, probably due to the lack of variation for sensitivity to partial vernalization within these materials. These results are in agreement with those of Loskutov (2001), who found that heading date was more influenced by cold requirement than daylength sensitivity. However, the lack of differentiation observed in the data could also be related to a low photoperiodic difference between the studied sites, given that at the beginning of March, the photoperiod has the same value for latitudes between 40°N and 50°N (see Fig. 3B). This suggests that local variation in ear emergence during this period should be mainly due to differences in temperature rather than to differences in daylength (Cooper, 1952). Moreover, in the present study, daylength sensitivity was estimated based on field and nursery heading date records, which might be inappropriate. Consequently, to confirm the results, this character should be measured in a growth chamber, where photoperiod is artificially controlled.

The DM system appeared to be able to maintain diversity for two out of three components of flowering time. For these traits, as previously described for other characters, the loss of variability within each population was compensated by differentiation between populations. Local adaptation to each contrasted environment was in progress. Thus, provided the sites chosen for growing populations reflect the range of environmental conditions addressed by a breeding programme, this strategy appeared promising for providing pre-breeding material adapted to specific geographical areas and agricultural environments.

Furthermore, the results of this study led us to wonder about the genetic changes underlying the phenotypic differentiation found between the DM populations. Associations between a latitudinal cline in earliness and allelic variation have already been shown for photoperiod sensitivity in *Populus* (Howe *et al*., 1998) or vernalization requirement in *Beta maritima* (Van Dijk *et al*., 1997; Boudry *et al*., 2002). In wheat, mapping and segregation analyses have shown that vernalization requirement and photoperiod sensitivity are regulated mainly by four major genes (*Vrn-A1*, *Vrn-B1*, *Vrn-D1* and *Vrn4*) and by three major genes (*Ppd-A1*, *Ppd-B1* and *Ppd-D1*), respectively (Pugsley, 1971, 1972; Welsh *et al*., 1973; Law *et al*., 1976; Law, 2000), whereas narrow-sense earliness is more polygenic. Recent advances in molecular biology and genomics provide efficient tools for detecting and isolating these genes (Blàsquez, 2000), as recently demonstrated by Yan *et al*. (2003, 2004). Thus, analysis of allelic variation and polymorphism of these genes in DM populations will allow a better understanding of the genetic adaptation of the DM populations to their growing sites and, more generally, elucidation of some of the genetic mechanisms involved in adaptive responses to climatic conditions in wheat.

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